Supplementary information

Linking performance and microbial ecology in a biological sulphate reducing reactor system developed for the treatment of acid rock drainage

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Figure S1 The composition of the inoculum show at (A) phylum and class resolution and the (B) dominant OTUs shown as a rank abundance curve. The inoculum was sampled in duplicate and these samples sequenced in parallel. Error bars represent one standard deviation from the mean ($n=2$).

Figure S2 Rank abundance curves of the (A) associated, (B) attached and (C) planktonic microbial communities isolated from the effluent zone of the LFCR at steady-state a one-day HRT. These communities were sampled in duplicate and these samples sequenced in parallel. Error bars represent one standard deviation from the mean ($n=2$).

Table S1 Performance and physiochemical data associated with the acetate-supplemented LFCR effluent zone at increasing applied dilution rates. Single standard deviations (SD) from the mean were calculated from a minimum of four samples taken during a steady state of at least three HRT.

dilution rate (h ⁻¹)	HRT (days)	VSLR (mg/L.h)	residual sulphate (mg/L)	(± SD)	VSRR (mg/L.h)	(± SD)	sulphide (mg/L)	(± SD)	residual acetate (mg/L)	(± SD)	predicted acetate (mg/L)	ph	(± SD)	redox potential (mV)	(± SD)
0.010	4	10.4	312.4	27.7	7.16	0.20	212.2	27.9	643.0	50.4	555.4	7.51	0.0	-379.0	18.4
0.014	3	13.9	530.1	14.5	6.53	0.20	166.9	8.9	797.1	77.2	689.2	7.49	0.2	-389.0	0.0
0.016	2.66	15.7	557.8	29.7	6.93	0.47	160.2	13.9	775.8	63.3	706.2	7.43	0.1	-389.0	9.9
0.018	2.33	17.9	607.5	14.7	7.02	0.47	150.8	3.4	670.0	19.3	736.8	7.32	0.0	-375.5	2.1
0.021	2	20.8	422.4	13.2	12.03	0.58	216.1	5.2	590.4	11.8	623.0	7.43	0.1	-385.5	0.7
0.028	1.5	27.8	472.9	30.9	14.64	0.86	184.3	2.7	712.7	19.5	654.1	7.31	0.2	-356.7	11.9
0.032	1.3	32.1	515.1	28.9	15.54	0.86	168.3	9.7	661.7	12.6	680.0	7.48	0.1	-357.5	23.7
0.042	1	41.7	589.1	22.7	17.81	0.00	138.0	8.3	682.2	14.1	725.5	7.37	0.1	-341.5	0.7

Table S2 Biomass retained by the acetate-supplemented LFCR at a four-day and one-day HRT. This includes biomass physically attached to the carbon fibre support structures, the microbial phase associated with this biofilm but not physically attached, and free-floating planktonic cells. Single standard deviations (SD) from the mean were calculated from duplicate cell counts performed on samples isolated in duplicate.

dilution rate (h ⁻¹)	HRT (days)	Reactor zone	Attached (cells/mL)	(± SD)	Associated (cells/mL)	(± SD)	Planktonic (cells/mL)	(± SD)
0.010	4	inlet zone	2.58E+09	9.62E+08	4.66E+08	5.65E+07	2.55E+08	8.84E+06
0.010	4	effluent zone	2.88E+09	2.90E+08	3.79E+08	3.47E+07	2.44E+08	1.99E+07
0.042	1	inlet zone	1.74E+09	2.65E+08	1.33E+08	2.86E+07	1.66E+08	1.33E+07
0.042	1	effluent zone	2.07E+09	3.02E+08	1.81E+08	5.60E+07	1.53E+08	4.42E+06

Table S3 The planktonic cell concentrations supported by the acetate-supplemented LFCR at increasing applied dilution rates. Single standard deviations (SD; ±) from the mean were calculated from duplicate cell counts performed on samples isolated in duplicate.

dilution rate (h ⁻¹)	HRT (days)	Effluent zone (cells/mL)	(± SD)	Inlet zone (cells/mL)	(± SD)	Average across reactor (cells/mL)	(± SD)
0.010	4	2.55E+08	1.99E+07	2.44E+08	8.84E+06	2.49E+08	1.44E+07
0.014	3	2.02E+08	1.55E+07	1.67E+08	2.21E+06	1.84E+08	8.84E+06
0.016	2.66	1.67E+08	2.43E+07	1.98E+08	2.87E+07	1.83E+08	2.65E+07
0.018	2.33	1.88E+08	1.33E+07	2.48E+08	2.87E+07	2.18E+08	2.10E+07
0.021	2	2.28E+08	2.21E+07	2.95E+08	1.55E+07	2.62E+08	1.88E+07
0.028	1.5	2.42E+08	2.21E+06	2.56E+08	2.21E+07	2.49E+08	1.22E+07
0.032	1.3	2.03E+08	4.42E+06	2.22E+08	2.21E+07	2.13E+08	1.33E+07
0.042	1	1.66E+08	4.42E+06	1.53E+08	1.33E+07	1.59E+08	8.84E+06

Table S4 Relative abundance of taxonomic classification of the 58 most abundant OTUs in the LFCR effluent and inlet zone microbial communities sampled during the HRT study.

Phylum	Family	OTU	attached				associated				planktonic							
			4		1		4		1		4		3		1.5		1	
			effluent zone	inlet zone	effluent zone	inlet zone	effluent zone	inlet zone	effluent zone	inlet zone	effluent zone	inlet zone	effluent zone	inlet zone	effluent zone	inlet zone	effluent zone	inlet zone
Bacteroidetes	Flavobacteriaceae	Lutaonella (0)	45.00	45.45	28.68	26.08	29.25	34.53	18.71	17.87	1.04	0.83	0.91	0.88	1.12	0.35	0.38	0.11
Firmicutes	Carnobacteriaceae	Trichococcus (33)	0.01	0.00	2.97	15.78	0.00	0.00	25.91	3.56	0.00	0.00	0.00	0.00	0.95	0.53	20.63	33.49
Bacteroidetes	Flavobacteriaceae	Pustulibacterium (3)	1.75	1.79	0.25	0.38	6.21	5.29	0.14	0.43	28.65	27.59	28.48	30.79	10.02	7.00	0.08	0.08
Synergistetes	Synergistaceae	Aminomonas (2)	1.92	1.83	1.44	1.27	3.33	3.33	1.21	3.47	7.15	6.35	23.43	20.19	4.91	6.74	1.93	1.42
Bacteroidetes	Porphyromonadaceae	Macellibacteroides (9)	0.07	0.04	0.13	0.11	0.34	0.28	0.47	0.45	1.42	1.39	1.83	2.02	12.23	23.19	2.83	3.31
Proteobacteria	Desulfomicrobiaceae	Desulfomicrobium (1)	0.02	0.03	9.12	6.05	0.30	0.32	2.70	15.65	1.09	1.03	1.02	1.02	10.12	5.25	5.49	1.39
Proteobacteria	Desulfobacteraceae	Desulfobacter (18)	0.27	0.51	10.40	6.09	1.41	1.02	13.60	11.53	0.06	0.10	0.11	0.08	9.33	7.60	1.22	0.96
Bacteroidetes	Marinilabiliaceae	Labilibacter (62)	0.02	0.05	0.14	0.11	0.12	0.10	0.11	0.20	1.49	1.49	7.95	12.98	0.42	0.40	0.04	0.03
Synergistetes	Synergistaceae	Dethiosulfovibrio (13)	0.76	0.83	0.72	0.41	2.18	1.91	2.19	3.84	3.01	2.57	0.35	0.24	12.36	8.98	11.13	7.20
Proteobacteria	Desulfobacteraceae	Desulfatitalea (177)	9.55	8.43	0.57	1.27	7.71	6.52	1.20	0.46	0.02	0.01	0.00	0.00	0.02	0.01	0.05	0.02
Bacteroidetes	Lentimicrobiaceae	Lentimicrobium (41)	9.33	6.19	2.78	2.92	6.72	6.73	2.14	1.46	1.14	1.22	0.63	0.63	1.95	0.80	0.13	0.02
Bacteroidetes	Bacteroidaceae	Bacteroides (23)	0.01	0.01	0.04	0.05	0.02	0.03	0.28	0.40	0.33	0.50	2.22	2.60	4.34	8.40	4.48	6.58
Chlorobi	Chlorobiaceae	Chlorobium (40)	0.53	0.42	7.29	0.23	0.52	0.40	0.27	1.21	0.16	0.12	0.09	0.12	0.36	0.38	0.36	0.02
Synergistetes	Synergistaceae	Dethiosulfovibrio (10)	2.42	1.99	0.48	0.20	5.34	4.33	0.65	0.50	6.72	5.63	1.60	1.40	0.59	0.39	0.36	0.24
Proteobacteria	Desulfovibrionaceae	Desulfovibrio (6)	0.25	0.45	0.14	0.28	0.44	0.32	0.22	0.19	5.96	6.57	2.77	1.98	0.26	0.42	0.50	0.25
Proteobacteria	Enterobacteriaceae	Enterobacter (16)	0.01	0.02	0.17	0.10	0.04	0.02	0.30	0.39	0.07	0.09	3.45	2.73	4.01	3.51	5.17	5.96
Proteobacteria	Halothiobacillaceae	Halothiobacillus (60)	0.32	0.58	0.16	5.93	0.90	1.74	0.09	0.83	0.08	0.12	0.00	0.00	0.04	0.02	0.06	0.02
Proteobacteria	Geobacteraceae	Geobacter (104)	0.92	1.49	0.13	1.87	1.11	2.00	0.01	0.20	5.61	5.65	0.15	0.15	0.08	0.07	0.09	0.01
Firmicutes	Ruminococcaceae	Anaerotruncus (69)	0.00	0.02	0.05	0.05	0.08	0.02	0.11	0.24	0.12	0.21	0.10	0.07	0.92	1.73	4.42	5.38
Bacteroidetes	Bacteroidaceae	Bacteroides (83)	0.01	0.01	0.06	0.07	0.11	0.07	0.09	0.38	2.48	2.65	3.49	2.83	0.73	0.96	4.09	4.92
Bacteroidetes	Dysgonamonadaceae	Proteiniphilum (29)	0.98	1.14	0.37	0.34	2.01	3.44	0.21	0.29	3.61	4.86	1.07	0.77	1.08	0.39	2.13	1.85
Verrucomicrobia	unclassified	Verrucomicrobia (67)	0.23	0.22	1.21	0.18	1.21	0.99	0.24	0.82	4.80	3.81	0.32	0.34	0.38	0.19	0.13	0.04
Bacteroidetes	Paludibacteraceae	Paludibacter (36)	0.12	0.14	1.79	1.15	0.26	0.15	2.75	4.13	0.52	0.67	0.12	0.08	0.97	0.53	1.52	1.02
Bacteroidetes	Flavobacteriaceae	Lutaonella (68)	1.48	1.48	0.25	0.16	2.78	2.08	0.11	0.04	4.19	4.36	0.06	0.07	1.13	0.82	0.03	0.01
Firmicutes	Peptococcaceae	Sporotomaculum (112)	1.74	2.20	0.66	0.61	4.34	3.11	0.45	0.24	2.01	2.27	0.13	0.24	0.06	0.04	0.00	0.01
Thermotogae	Petrotogaceae	Geotoga (34)	1.18	2.04	1.84	2.48	0.98	1.23	1.16	3.80	0.41	0.43	0.78	1.05	0.66	0.66	2.04	1.61
Proteobacteria	Campylobacteraceae	Arcobacter (5)	0.02	0.00	0.02	0.04	0.01	0.01	0.02	0.03	0.09	0.06	0.01	0.03	0.33	0.58	2.01	3.49
Bacteroidetes	Dysgonamonadaceae	Dysgonomonas (43)	0.12	0.09	0.10	0.16	0.29	0.08	0.22	1.07	2.36	2.62	0.46	0.38	1.03	1.04	3.28	3.07
Bacteroidetes	Sphingobacteriaceae	Anseongella (45)	0.01	0.01	0.02	0.05	0.10	0.05	0.03	0.13	2.68	2.92	1.83	1.49	2.60	2.07	0.24	0.10
Proteobacteria	Helicobacteraceae	Wolinella (28)	0.00	0.00	0.10	0.08	0.00	0.01	0.02	0.18	0.02	0.03	0.01	0.03	0.11	0.21	2.59	2.87
Firmicutes	Bacillaceae	Bacillus (32)	0.08	0.03	0.09	0.03	0.11	0.14	0.07	0.06	0.03	0.04	0.56	0.52	2.25	2.74	0.00	0.00
Synergistetes	Synergistaceae	Aminivibrio (54)	2.74	1.94	0.76	0.90	2.69	1.55	1.05	1.00	1.05	0.89	1.41	1.06	0.65	0.34	0.36	0.14
Proteobacteria	Rhodospirillaceae	Aliidongia (52)	0.46	0.70	2.72	2.09	0.31	0.36	1.90	1.42	0.28	0.20	0.32	0.21	0.10	0.10	0.09	0.04
Synergistetes	Synergistaceae	Aminobacterium (70)	1.59	1.96	0.89	2.66	1.01	1.39	1.34	0.75	0.04	0.02	0.01	0.01	0.00	0.01	0.01	0.01
Proteobacteria	Desulfobulbaceae	Desulfobulbus (58)	1.46	2.44	0.05	0.39	1.83	1.85	0.06	0.04	0.03	0.05	0.09	0.02	0.02	0.01	0.07	0.02
Firmicutes	Peptococcaceae	Pelotomaculum (100)	0.16	0.09	2.41	0.87	0.15	0.06	0.62	1.14	0.04	0.02	0.07	0.04	0.18	0.08	0.07	0.03
Firmicutes	Lachnospiraceae	Anaerotignum (21)	0.05	0.10	0.09	0.06	0.16	0.14	0.35	0.49	1.06	1.64	0.31	0.37	0.58	0.66	2.17	2.17
Proteobacteria	Campylobacteraceae	Sulfurospirillum (57)	0.00	0.00	0.09	0.23	0.01	0.01	0.12	0.30	0.01	0.02	0.07	0.06	0.31	0.54	2.02	1.55
Synergistetes	Synergistaceae	Lactivibrio (56)	0.87	0.71	1.18	0.80	0.63	0.81	1.88	0.98	0.17	0.12	0.05	0.04	0.44	0.10	0.13	0.02
Proteobacteria	Campylobacteraceae	Arcobacter (285)	0.45	0.34	0.00	0.00	1.72	0.98	0.00	0.00	0.08	0.06	0.00	0.00	0.00	0.00	0.00	0.00
Proteobacteria	Desulfarculaceae	Desulfarculus (97)	0.81	1.44	0.16	0.57	1.68	1.53	0.19	0.09	0.17	0.32	0.03	0.06	0.04	0.02	0.00	0.00
Firmicutes	Ruminococcaceae	Pseudoflavonifractor (98)	0.01	0.02	0.09	0.11	0.08	0.05	0.35	0.36	0.91	1.16	1.67	1.22	0.89	0.91	1.58	1.34

Table S5 The variation in the relative abundance of OTUs found in the inlet and effluent zones of the LFCR metagenomes, sampled at the same HRT and from the same microbial phase, shown as mean and median coefficients of variation (SD / mean).

	attached				associated				planktonic							
	4		1		4		1		4		3		1.5		1	
	effluent zone	inlet zone	effluent zone	inlet zone	effluent zone	inlet zone	effluent zone	inlet zone	effluent zone	inlet zone	effluent zone	inlet zone	effluent zone	inlet zone	effluent zone	inlet zone
median coefficient of variation (%)	21.7		22.5		13.6		30.0		11.7		10.3		25.0		20.9	
mean coefficient of variation (%)	28.0		31.6		17.7		34.3		12.3		14.0		24.1		32.0	

Table S6 Relative abundance of the predominant microbial classes identified in the LFCR effluent zone microbial communities during the HRT study.

Phyla	Class	4-day HRT			3-day HRT	1.5-day HRT	1-day HRT		
		attached	associated	planktonic	planktonic	planktonic	planktonic	attached	associated
Proteobacteria	Deltaproteobacteria	16.26	14.75	13.99	3.76	13.70	2.83	15.77	30.53
Proteobacteria	Alphaproteobacteria	0.85	0.48	0.23	0.35	0.78	0.18	2.37	1.54
Proteobacteria	Gammaproteobacteria	0.68	1.77	0.21	3.24	4.38	7.02	6.93	1.70
Proteobacteria	Epsilonproteobacteria	0.37	1.00	0.17	0.12	1.36	8.00	0.36	0.51
Bacteroidetes	Flavobacteriia	48.72	41.91	32.78	32.05	8.18	0.20	26.64	18.36
Bacteroidetes	Bacteroidia	8.51	11.70	15.61	23.56	37.03	22.04	6.60	10.27
Bacteroidetes	Sphingobacteriia	0.01	0.05	2.92	1.49	2.16	0.24	0.25	0.34
Firmicutes	Bacilli	0.03	0.14	0.04	0.54	3.29	33.65	15.84	3.64
Firmicutes	Clostridia	4.33	4.66	6.81	5.60	6.53	11.61	8.46	8.63
Chlorobi	Chlorobia	0.42	0.40	0.12	0.12	0.38	0.02	0.23	1.21
Spirochaetes	Spirochaetia	0.88	1.03	1.47	1.61	0.90	0.75	1.51	2.14
Synergistetes	Synergistia	9.81	14.40	16.22	23.23	16.92	9.23	8.22	12.28
Thermotogae	Thermotogae	2.07	1.24	0.43	1.05	0.66	1.62	2.52	3.80
Verrucomicrobia	unclassified	0.22	0.99	3.81	0.71	0.27	0.10	0.19	0.87
Other	Other	6.84	5.47	5.19	2.59	3.45	2.51	4.12	4.19

Table S7. Diversity statistics describing the characterised reactor and inoculum metagenomes.

Sample origin	HRT (days)	Phase	OTUs	Chao1	Shannon	Inverse Simpson	Goods Coverage
Inoculum	N/A	N/A	180	209.5	4.531	0.920	0.9982
Effluent zone	4	Attached	182	211.1	3.745	0.776	0.9982
Inlet zone	4	Attached	206	231.1	3.876	0.778	0.9983
Effluent zone	4	Associated	202	224.0	4.536	0.890	0.9985
Inlet zone	4	Associated	190	263.9	4.333	0.861	0.9974
Effluent zone	4	Planktonic	162	197.2	4.374	0.891	0.9972
Inlet zone	4	Planktonic	158	174.7	4.450	0.898	0.9979
Effluent zone	3	Planktonic	254	309.8	4.045	0.852	0.9977
Inlet zone	3	Planktonic	277	349.0	3.924	0.843	0.9981
Effluent zone	1.5	Planktonic	274	319.0	4.792	0.932	0.9976
Inlet zone	1.5	Planktonic	249	299.1	4.542	0.909	0.9976
Effluent zone	1	Planktonic	262	359.8	4.870	0.927	0.9975
Inlet zone	1	Planktonic	196	263.0	4.116	0.863	0.9970
Effluent zone	4	Attached	182	211.1	3.745	0.776	0.9982
Inlet zone	4	Attached	206	231.1	3.876	0.778	0.9983
Effluent zone	1	Associated	296	328.2	5.000	0.930	0.9981
Inlet zone	1	Associated	301	330.3	5.022	0.921	0.9987